



St. Paul Hospital Millennium Medical College

Post Graduate Study Program

**Breast Cancer Biomarker Status and Histo-Pathologic Correlation in
St. Paul Hospital Millennium Medical College, Addis Hiwot General
Hospital and Onco-pathology Diagnostic Center, Addis Ababa,
Ethiopia.**

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Addis Ababa, Ethiopia,

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Abstract

Background: By using immunohistochemical biomarker (estrogen receptor, progesterone receptor and human epidermal receptor-2) breast cancer is classified into 4 major molecular subtypes, namely luminal A, luminal B, triple negative and human epidermal receptor-2 overexpressing. Triple negative breast carcinoma is a heterogeneous group that includes tumors with good as well as bad prognosis. Luminal A tumors are the most common type, tend to have better prognosis. Triple negative and human epidermal receptor-2 overexpressing type breast carcinoma has the worst prognosis. Hormone receptor positive breast cancers are tend to be diagnosed in older patients, lower grade and stage compared to hormone receptor negative carcinomas.

Objective: To study breast cancer immunohistochemical biomarker and histo-pathologic correlation at Saint Paul Hospital Millennium Medical College, Addis Hiwot General Hospital, and Onco-pathology cancer diagnostic center Addis Ababa, Ethiopia from July 2021 to July 2024.

Materials and methods: A medical record review was conducted using data from the from October 2023 to October 2024 at St. Paul hospital millennium medical college, Addis Hiwot General Hospital and onco-pathology diagnostic center. Data was collected using data extraction checklist by data collectors and principal researcher from St. Paul Hospital millennium medical college patient charts, and data base of Addis Hiwot General Hospital and Onco-pathology Diagnostic Center. Data was analyzed by the SPSS version 27. Descriptive statistics like frequencies and cross tabulation were made for most selected variables. Chi square test and multinomial logistic regression analysis was used to assess the association between the dependent and independent variables.

Results: Among the 490 breast cancer cases majority (68.18%) were below the age of 50 yrs. The most common histologic subtype was invasive carcinoma of NST(83.6%) followed by mixed carcinoma of invasive lobular and ductal carcinoma. Majority of cases were positive for ER (82.3%) and PR (72.8%) and 75.2% of cases were HER-2 negative. Luminal A accounts for 57.9% cases followed by luminal B (26.2%). Triple negative breast carcinoma was the least common subtype which accounted for 7.37%. Age, tumor size and Nottingham grade have

shown significant association with the intrinsic subtype of breast carcinomas. HER-2 positive breast cancer tends to occur in younger age and to have larger tumor size

Conclusion: The mean age at diagnosis of breast cancer was 45 years. Invasive carcinoma of NST was the most common histologic subtype. Hormone receptor status expression was positive in majority of breast cancer cases. Luminal A was the most common intrinsic subtype whereas triple negative was the least. HER-2 positive breast cancer tends to occur in younger age and larger tumor size.

Key words: Breast cancer, Histo-pathology, Hormone receptor, Breast biomarker

ABBREVIATIONS AND ACRONYMS

BLBC-Basal Like Breast Carcinoma

ER-Estrogen Receptor
 ErbB2-Erythroblastic oncogene B2
 ERP - Estrogen Receptor Positive
 ERP - Estrogen Receptor Negative
 HR-Hormone receptor
 HDI- Human Development Index
 HER-2- Human Epidermal Growth Hormone Receptor-2
 IHC-Immunohistochemical
 IRB- Institutional review board
 NST- No special type
 PR-Progesterone Receptor
 PRP- Progesterone Receptor Positive
 PRN- Progesterone Receptor Negative
 PI3K-Phosphoinositide 3-kinase
 SPHMMC- Saint Paul Hospital Millennium Medical College
 USA- United States of America

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1. INTRODUCTION

1.1 BACKGROUND

Breast cancer is a disease in which abnormal breast cells grow out of control and form tumors. Breast cancer cells begin inside the milk ducts and/or the milk-producing lobules of the breast(1).

Certain factors increase the risk of breast cancer including increasing age, obesity, harmful use of alcohol, family history of breast cancer, history of radiation exposure, reproductive history (such as age that menstrual periods began and age at first pregnancy), tobacco use and postmenopausal hormone therapy. Approximately half of breast cancers develop in women who have no identifiable breast cancer risk factor other than gender (female) and age (over 40 years)(1).

An estimated 2.3 million females were diagnosed with breast cancer in 2020, accounting for approximately 24.5% of all cancer cases worldwide. There were approximately 685,000 females who died from breast cancer in 2020, accounting for approximately 15.5% of all cancer deaths in the world(2). Among women, breast cancer accounts for 1 in 4 cancer cases and for 1 in 6 cancer deaths.(3)

It's also the most common cancers among females in Ethiopia accounting for 33% of all female cancer cases(3).

Global estimates reveal striking inequities in the breast cancer burden according to human development. For instance, in countries with a very high Human Development Index (HDI), 1 in 12 women will be diagnosed with breast cancer in their lifetime and 1 in 71 women die of it. In contrast, in countries with a low HDI; while only 1 in 27 women is diagnosed with breast cancer in their lifetime, 1 in 48 women will die from it(1).

In sub-Saharan Africa there is a rapid increase in the incidence of breast cancer. Mortality rates in sub-Saharan African regions have increased simultaneously and rank now in the world highest, reflecting weak health infrastructure and subsequently poor survival outcomes. In sub-

Saharan Africa, breast cancer is responsible for one in four diagnosed cancers and one in five cancer deaths in women (3)

Breast cancer was historically perceived as one disease with varying histopathological features and response to systemic treatment(4). However currently it's identified as a heterogeneous disease morphologically as well as at molecular level; hence, existing histological classifications do not fully capture the varied clinical course and prognosis of this disease. Histological type, grade, tumor size, lymph node involvement, and estrogen receptor, human epidermal growth receptor-2 status and molecular phenotype all influence prognosis and the probability of response to systemic therapies (5). Histologic type of invasive breast carcinoma includes infiltrating carcinoma of NST, invasive lobular, mucinous (colloid), tubular, medullary, papillary and mixed carcinoma. Of these, invasive carcinoma of NST is by far, the most common subtype accounting for 70–80% of all invasive lesions(6)

Global gene expression profiling studies classified breast cancers into 4 major intrinsic subtypes namely luminal A, luminal B, HER2-overexpressing and triple negative. These studies also demonstrated the relevance of the immunophenotypic classification by hormone receptors and HER2 status. ER expression stratifies breast cancers into 2 distinct clusters: ER positive and ER negative. Luminal A and B subtypes were enriched with ER-positive cancers, whereas HER2-overexpressing and triple negative breast cancers were ER negative. Apart from the expression of ER and ER-related genes, different subtypes showed differential expression of genes related to proliferation and HER2 amplicon. These different intrinsic subtypes revealed differences in incidence, clinical and pathological features, and to a large extent, overlapped with the established clinical and histopathologic classifications(7).

Of the intrinsic subtype of breast carcinoma Luminal A tumors are the most common type, tend to grow at a slower rate than other cancer types. They are also called hormone receptor-positive because they're defined by their hormone receptors. They are positive for ER and PR. But HER-2 is negative. A cancer that is estrogen receptor- and/or progesterone receptor positive grows from estrogen and/or progesterone so, drugs that lower the amount of these hormones tend to be

useful in treating this type of breast cancer. The second common type are Luminal B breast cancer which tends to grow faster than the luminal A type and are considered more aggressive. They are positive for hormone receptors and for a higher-than-normal amount of human epidermal receptor 2, so they're both hormone receptor positive and human epidermal receptor 2-positive. On the other hand, the cells of triple negative breast cancer type do not contain receptors for estrogen, progesterone or HER2 and the prognosis with this type of breast cancer is very poor. The last one is HER2-positive cancers which are ER- and PR-negative and human epidermal growth factor receptor 2 (HER2)-positive.(8)

Luminal A type carcinomas include invasive carcinoma of no special type grade 1 or 2, tubular, mucinous, invasive lobular carcinoma (classic and variants), cribriform, invasive carcinoma with neuroendocrine differentiation, micropapillary carcinomas. Luminal B: include invasive carcinoma of no special type grade 2 or 3. Triple negative includes high and low grade tumors. High grade TNBC: invasive carcinoma of no special type grade 3, spindle cell metaplastic breast carcinoma, medullary carcinoma, apocrine carcinoma. Low grade TNBC includes salivary gland-like tumors (secretory carcinoma, adenoid cystic carcinoma, acinic cell carcinoma, mucoepidermoid carcinoma), tall cell carcinoma with reverse polarity, low grade metaplastic carcinomas. HER2 enriched predominantly consists invasive carcinoma of no special type, high histological grade.(9)

Biological pathways show reasonably good performance in differentiating good and bad survival outcomes, and likewise molecular subtyping of tumors. There is thus considerable association between molecular and clinical features. In fact, the four molecular subtypes are known to associate with distinct survival outcomes with basal-like and HER2 tumors characterized by high aggressiveness and low survival, compared with luminal-B and luminal-A tumors. This study also discussed genes that show significant degree of differentiation between different drive prognosis. PI3K/RAS are frequently over-expressed in aggressive breast tumors, especially basal-like, whereas cell cycle genes are markers of high proliferative rate in tumors(10).

1.2 Statement of the problem

The capacity to forecast a response to more recent targeted therapies has been partially addressed by the discovery of the molecular subtypes of breast cancer. Notably, the basal like/triple-negative (ER-/PR-/ErbB2-) subtype has the shortest survival time among the molecular subtypes in terms of predicting overall survival as well as disease-free survival. These differences are highly significant. It is reasonable to anticipate that the use of molecular subtyping in clinical practice will yield valuable data about the likelihood of a pathological complete response, risk of relapse, and prognosis specific to each patient (11).

In contrast to the Western world, where the prevalence of hormone receptor-positive breast cancer is approximately 70%, reported rates of both PR and ER negativity in Africa have been found to vary significantly across studies, ranging from 30–87 percent and 36–79 percent, respectively. Although HER2 status has not been extensively studied, tumors have generally been categorized as not expressing this marker. Because of this, the incidence of triple negative cancers has been elevated; several studies indicate that most African women receive a diagnosis of these tumors (12).

Understanding tumor characteristics, particularly hormone receptor expression, in the adjuvant and metastatic contexts is essential for adhering to standards of care for breast cancer. Clinicians in Africa frequently treat patients with breast cancer with hormonal therapy alone, typically tamoxifen, without knowing the patient's hormone receptor status due to resource constraints and the comparatively high cost of receptor testing. Thus, it's likely that Africans who are afflicted with breast cancer don't receive the kind of effective systemic therapy that has been recommended as cited by (12).

Research conducted in Ethiopia has revealed inconsistent results about the most prevalent intrinsic subtypes and their relationship to hormone receptor status, as well as variable rates of hormone receptor status. Luminal A is the most prevalent intrinsic subtype in the majority of studies conducted in our nation. Nonetheless, other studies have indicated that triple negative

carcinoma is the most prevalent type. Reports of triple negative breast cancer range from 23 to 33.1%, while those of luminal A breast cancer range from 25 to 40% in various studies (13–16).

1.3 Significance of the study

Although ER, PR and HER-2 identification is very crucial in delivering appropriate treatment, literatures are mentioning that in Africa breast cancer patients are being treated with hormonal therapy without knowing their status. Furthermore, in a resource limited settings like Ethiopia, there are paucity of researches that are done so far and some these weren't recent and others showed conflicting results regarding hormone receptor status and correlation between intrinsic subtypes and histopathologic findings. Therefore, a detailed study has to be done to have local receptor pattern understanding and thus establishing effective treatment strategies.

2. LITERATURE REVIEW

In a study of 334 breast cancer patients from different hospitals in Addis Ababa, Ethiopia 104 samples (31.1%) were luminal A, 91 samples (27.2%) were luminal B, 62 samples (18.6%) were HER2-enriched and 77 samples (23.1%) were basal-like. The intrinsic subtypes were found to be associated with clinical and histopathological parameters such as steroid hormone receptor status, HER2 status, Ki-67 proliferation index and tumor differentiation, but not with age, tumor size or histological type. (13)

Out of 201 cases examined in four different regions of Ethiopia between 2015 and 2021, half of the specimens tested negative for ER and PR. HER2 positive cases made up 22% of the total. The presence of an ER-positive tumor with a histological grade I or II was 2.9 times more common than that of a grade III tumor ($p = 0.005$) in this study, which included 161 breast cancer cases in multiple logistic regression analysis. There seems to be a 2.1 increase in the risk of ER-positive breast cancer in older women (>50 years vs. Less than 50 years) ($p = 0.039$). Large (T3 or T4) tumor sizes were 4.1 times more common in HER2-positive tumors than in HER2-negative tumors ($p = 0.007$). The likelihood of HER2 positive breast cancer cases in eastern Ethiopia (Harer) was 3.6 times higher than that of cases in northern Ethiopia (Mekele). Forty-three percent of breast cancer cases had Ki-67 scores of ≥ 20 percent. The multiple logistic regression analysis of 149 breast cancer patients revealed that the likelihood of having Ki-67 ≥ 20 was 6.4 times higher in eastern Ethiopia (Harer) compared to northern Ethiopia (Mekele). (15)

The distribution of IHC subtypes in 163 samples with all available IHC was found to be 33.1% TNBC, 27.6% luminal B, 25.2% luminal A, and 14.1 percent HER2 enriched in univariate analysis of the above study. Luminal A subtypes were 10.4 times more likely to have histological grade I or II than grade III among the 131 BC patients included in the multiple logistic regression analysis ($p = 0.002$). In comparison to northern Ethiopia (Mekelle), the luminal A subtype of BC was three times more common in southern Ethiopia (Hawassa) ($p = 0.109$). According to the

study, HER2 enriched subtypes were 4 points higher in cases with tumor sizes T3 or T4 than in tumor sizes T1 or T2. The greatest percentage of cases from southwestern Ethiopia (Jimma) (37.5 percent) and northern Ethiopia (Mekele) (36.4 percent) were found to have TNBC in the univariate analysis. After adjusting for other variables, a multiple logistic regression model showed that TNBC was 2 points more likely in Jimma, in the southwest of Ethiopia, than it was in Mekelle, in the north of the country. However, this difference was not statistically significant.(15)

In research done in Kijabe, East Africa from 2001-2007, ER/PR testing was done in 120: 24% had ER-positive tumours, 34% were ER- and/or PR-positive, 10% were ER-negative but PR-positive tumours, and 66% were negative for ER and PR. ER/PR positivity was not associated with stage ($P = 0.28$) and was not related to age, parity, menopausal status, or node metastases. Increasing tumour grade was associated with PR expression ($P = 0.02$) with decreasing frequency of PR positive tumours as histological grade increased; there was weak evidence of an association between grade and ER expression ($P = 0.06$). Of cases tested, 26.5% overexpressed Her2.(17)

In a meta-analysis done on five sub-Saharan countries including Ethiopia, Kenya, Nigeria, Rwanda, and Uganda, from 1998-2018, the mean age was 47.5 years, ER+ was the predominant BC receptor, 55%, overall, in the five East African countries, variability in the estimated pooled proportions of ER+ BC, ranging from 62% in Ethiopia to 42% in Uganda. The overall estimated proportion of HER2+ BC in this study was 23%, compared with under 15% in the United States. Overall estimated proportion of TNBC in this study was 27% with across-country variability from 21% to 35% in Ethiopia and Uganda, respectively. They also stated the lower proportion of TNBC in Kenya and Ethiopia needs further study as it could be linked to testing and/or biologic factors.(18)

In a scoping review and meta-analysis done in 24 African countries including Ethiopia where 63 studies were included from different regions, after analysis of the results, the pooled luminal frequency was 56.30% (95% CI, 51.88 to 60.67; $I^2 = 98\%$). When pooled by region, North Africa ($n = 21$) had the highest frequency of 63.42 (95% CI, 58.57 to 68.13; $I^2 = 97$). Central Africa also showed a high frequency of 81.89% but had representation from just two studies. The frequency of the luminal subtype was lowest in West Africa ($n = 15$) with 41.04% (95% CI, 31.27 to 51.17; $I^2 = 97$). Pooling by individual countries showed Kenya ($n = 3$) had the highest

frequency (68.44%; 95% CI, 62.65 to 73.96; $I^2 = 63\%$). Luminal frequency was lowest in Ghana (n = 2) with 27.46% (95% CI, 10.88 to 48.09; $I^2 = 94\%$). The level of between-study heterogeneity (I^2) was found to be high at 94%. The pooled HER2+ frequency was 12.61% (95% CI, 11.37 to 13.91; $I^2 = 86\%$). When pooled by region, West Africa (n = 19) had the highest frequency of 14.87 (95% CI, 11.52 to 18.56; $I^2 = 88\%$). It was lowest in Central Africa (n = 3) with 8.05% (95% CI, 2.33 to 116.31; $I^2 = 72$). Pooling by individual countries showed that Uganda (n = 3) had the highest HER2+ frequency (18.72%; 95% CI, 13.67 to 24.33; $I^2 = 34\%$). It was lowest in Kenya (n = 3) with 9.45% (95% CI, 7.81 to 11.23; $I^2 = 0\%$). The level of between-study heterogeneity (I^2) was found to be high at 86%. The pooled TNBC frequency was 28.10% (95% CI, 24.77 to 31.55; $I^2 = 97\%$). When pooled by region, West Africa (n = 19) had the highest frequency of 42.36 (95% CI, 35.88 to 48.97; $I^2 = 94\%$). It was lowest in Central Africa (n = 3) with 18.25% (95% CI, 7.53 to 32.08; $I^2 = 83$). Pooling by individual countries showed that Ghana (n = 3) had the highest TNBC frequency of 56.17% (95% CI, 49.77 to 62.47; $I^2 = 54\%$). It was lowest in Morocco (n = 7) with 15.52% (95% CI, 12.32 to 19.01; $I^2 = 91\%$). The level of between-study heterogeneity (I^2) was found to be high at 97% (19).

In the study done in the Saudi Arabia during 2012 to 2018 on a total of 740 breast cancer cases, the average age of the patient at time of diagnosis was 49 years. Most cases (n = 629, 85%) were ductal, a few of them (n = 84, 11.4%) were lobular, while the rest of the cases were of other histological types that included medullary, tubular, mucinous, metaplastic, adenoid cystic, and encysted papillary carcinoma. Positive ER immunostaining was found in 70.8% of the cases and the PR in 63.8%. HER2 immunostaining was found positive in 18.7% of the cases and equivocal in 22.8%. Luminal A was the most prevalent subtype (n = 434, 58.5%) followed by, triple negative (n = 117, 16%), luminal B (n = 104, 14%), and HER2-positive (n = 85, 11.5%). HER2 positive and Triple negative tumors showed tumor size >2cm, aggressive microscopic features and majority (2/3rd) demonstrated poorly differentiated carcinomas. HER2 positive tumors are least commonly associated with an in situ carcinoma.(20)

In a prospective study done in Iraq on 251 female patients from 2019 to 2021 the average age of cancer diagnosis was 48 years. Forty eight percent were having triple-negative breast carcinoma

and most of the participants with triple-negative were in the ≥ 70 age group followed by the 60–69 age group (55.26%) and 20–29 (50.0%). Only 7(2.78% of the patients showed all HR-positive status. Only 9.16% (23 of patients) had ER+ and PR +only. Most of them (15.78%) were in the age group 60–69. Seventeen percent had only HER2 positive.(21)

The Breast Pathology Study Group of the Chinese Society of Pathologists collected 12,467 IBC cases from 19 clinical centers in 2015 in China, of these cases 62.4% were ER⁺/PR⁺, 9.3% were ER⁺/PR⁻, 1.3% were ER⁻/PR⁺, and 27.0% were ER⁻/PR⁻. The assessment of HER2 status showed that the overall positive rate of HER2 (with a definition of immunohistochemistry [IHC] 3+ or IHC2+/*in situ* hybridization-positive) was 24.7% (range, 13.7%-35.7%) in each center.(22)

From 17 population-based cancer registries that participate in the SEER program in 2010, in USA from 50571 patients with known HR/HER2 status, 36810 (72.7%) were found to be HR+/HER2–, 6193 (12.2%) were triple-negative (HR–/HER2–), 5240 (10.3%) were HR+/HER2+, and 2328 (4.6%) were HR–/HER2+; 6912 (12%) of the case patients had an unknown HR/HER2 status. Incidence rates for HR+/HER2– were higher than those for other subtypes across all racial/ethnic groups and all age groups. Non-Hispanic white women had the highest rate for this subtype, followed by non-Hispanic black women, and then non-Hispanic Asian/Pacific Indian and Hispanic women. Non-Hispanic black women had the highest incidence rates of triple-negative breast cancer across all age groups. Patients with triple-negative, HR+/HER2+, and HR–/HER2+ breast cancer were 10% to 30% less likely to be diagnosed at older ages compared with HR+/HER2– patients and 6.4-fold to 20.0-fold more likely to present with high-grade disease. (23)

Based on the research published by American college of oncology on 200 female breast cancer cases in 2008 using clinicopathologic variables, young women illustrated lower estrogen receptor (ER) positivity ($P = .027$), larger tumors ($P = .012$), higher human epidermal growth factor receptor 2 (HER-2) overexpression ($P = .075$), lymph node positivity ($P = .008$), higher grade tumors ($P < .0001$), and trends toward inferior disease-free survival (DFS; hazard ratio = 1.32; $P = .094$). Using genomic expression analysis, tumors arising in young women had significantly

lower ER ($P < .0001$), and progesterone receptor (PR) expression ($P < .0001$), but higher HER-2 ($P < .0001$) and epidermal growth factor receptor (EGFR) expression ($P < .0001$). (24)

Among patients with known receptor status, 35% (95% CI, 32 to 38) of tumors were ERN, 47% (44 to 50) PRN, and 26% (23 to 29) HER2P. High concordance (82.2%) was found between ER and PR status: percentages jointly classified were ERP and PRP 50.2%, ERP and PRN, 14.9%, ERN and PRP 2.9% and ERN and PRN 32.0%; thus 68% of tumors were hormone receptor positive (ERP and/or PRP). ER, PR, and HER2 status were each missing for between 10% and 15% of women. The greatest ERN and PRN differences occurred with tumor grade: a fourfold greater risk of the tumor being ERN existed if it was grade 3 compared with grade 1. Higher grade was also, but less strongly, associated with an increased risk of HER2P status. Similar to tumor grade, stage III and IV tumors were almost 2 times more likely to be ERN and PRN, but stage was less strongly associated with HER2 status. Consequently, both higher grade and later stage were strongly associated with the combined subtype. HER2P-enriched (66%) and 58% of TRNs were diagnosed at stages III/IV compared with 47% of luminal A tumors. Both HER2P-enriched and TRN subtypes had >60% grade 3 tumors, compared with 28% of luminal A. Median age at diagnosis was youngest for luminal B tumors (49.6 years that is, >5 years younger than for luminal A tumors (55.0 years)). HER2P tumors were also diagnosed at younger ages than were TRN tumors. These arise from the trend of older age associated with a lower risk of being HER2P, whereas age was not strongly associated with ER or PR status.(25).

Studies of BC among international populations with African heritage have strengthened arguments that geographically defined ancestry influences the risk of TNBC. Immunohistochemistry resources are limited in the low and middle-income countries of Africa, and data on biomarker expression are often unavailable. However, investigators from various sites have reported elevated TNBC frequencies in comparison with the 15% prevalence observed in women of European ancestry, ranging from 23% to 82% of all cases. Most studies demonstrating higher frequencies of TNBC in Africa have represented western sub-Saharan Africa with less known about BC in east Africans. However, low frequencies of ER-negative BC have been reported in Ethiopia and in Ethiopian patients with BC residing in the United States. The first direct comparison of African American, white American, Ghanaian/West African and Ethiopian/East African patients with BC. This study found TNBC in 53.2% of Ghanaians and 29.8% of African Americans; the frequency of TNBC was similarly low among white Americans and Ethiopians, at 15.5% and 15.0% respectively ($P < .0001$) (26,27).

3. Objective

3.1 General objective

To study Breast cancer biomarker status and histo-pathologic correlation at Saint Paul Hospital Millennium Medical College, Addis Hiwot General Hospital, and Onco-pathology cancer diagnostic center, Addis Ababa, Ethiopia from July 2021 to July 2024.

3.2 Specific objective

To assess ER, PR, HER-2 and/or Ki-67 status of breast cancer at SPHMMC, Addis Hiwot General Hospital and Onco-pathology cancer diagnostic center over a specified period.

To identify the molecular subtypes of breast carcinoma.

To study the correlation between intrinsic subtypes of breast carcinoma and histo-pathologic feature

4. Methodology

4.1 Study Setting

St Paul's Hospital millennium medical college is one of the biggest Hospitals in Ethiopia, found in the capital Addis Ababa, Gulele sub-city near Swaziland Street. St Paul's Millennium Medical College, as it is known today, was established through a decree of the Council of Ministers in 2010, although the medical school opened in 2007 and the hospital was established in 1968 by the late Emperor Haile Selassie.

It is governed by a board under the Federal Ministry of Health. The College initiated Ethiopia's first integrated modular and hybrid problem-based curriculum for its undergraduate medical education, and is currently expanding to postgraduate and subspecialty programs.

St. Paul's Hospital Millennium Medical College provides healthcare and training to its students through its different biomedical and clinical departments, including Anatomy, Physiology, Biochemistry, Pharmacology, Public health, Pathology, General Surgery, Internal Medicine, Obstetrics and Gynecology, Pediatrics and child health, Emergency Medicine, Urology, Neurology, Orthopedics, Psychiatry, Ophthalmology, ENT, Dentistry and Maxillofacial Surgery, Radiology, Anesthesiology, Nursing, ICU (Intensive Care Unit), ART (HIV care), Endoscopy, Physiotherapy, Laboratory and Pharmacy. Pathology department is running residency program along with its vast pathologic services delivering 8845 biopsy, 4810 FNAC, 2800 hematopathology, 141 gynecologic cytology and 984 body fluid cytology cases over the last one year

Addis Hiwot General Hospital is a private hospital located in the center of Addis Ababa, at Bole Sub-city Wereda 4. It was established in 2000G.C (1993E.C), by three Ethiopian physicians and provides general, specialty and subspecialty services including oncologic services. It has established department of Anatomic pathology (AP), receive and analyze biopsies and all cytological samples.

Onco Pathology Diagnostic Center is the first standalone anatomic pathology laboratory established in May 2019 in Addis Ababa. It is an independent, pathologist-owned and managed pathology laboratory and practice, specializing in tissue pathology (histopathology). The lab is equipped with advanced instrumentation, including automated systems, and digital pathology scanners. It provides fine needle aspiration cytology, body fluid cytology, histopathology and immunohistochemical services.

4.2 Study Period

This study was conducted from November 1-30, 2024 G.C

4.3 Study Design

Medical record review was conducted in Saint Paul hospital millennium medical college, Addis Hiwot General Hospital and Onco-pathology Diagnostic Center from October 2023 to October 2024 G.C

4.4 Source Population

Source populations were all breast cancer diagnosed patients at SPHMMC, Addis Hiwot General Hospital and Onco-pathology Diagnostic Center.

4.5 Study Population

All breast cancer diagnosed patients from October 2023 to October 2024 in SPHMMC, Addis Hiwot General Hospital and Onco-pathology Diagnostic Center who fulfill inclusion criteria.

4.6 Selection criteria

4.6.2 Inclusion criteria

All breast cancer diagnosed patients over the past 1 year with histopathologic, ER, PR, HER- 2 and/or Ki-67 index results.

4.6.2 Exclusion criteria

All breast carcinoma cases with no immunohistochemical biomarker results and male patients with breast cancer were excluded. Recurrent breast cancer cases and breast cancer cases in SPHMMC with both biopsy and IHC results done at oncopathology were excluded in order to avoid data duplication.

4.7 Sample size

All breast cancer cases that fulfill the inclusion criteria were studied

4.8 Sampling procedure

The three institutions were selected for this study based on observational analysis on the pathologic service they are providing with in Addis Ababa, the capital city of Ethiopia

St.Paul Hospital Millennium medical College is one of the biggest tertiary care hospital in the country and the leading hospital providing well organized pathologic service and it accepts pathologic specimens of breast tissue from wide catchment areas along with its own vast service on breast surgery by breast and endocrine surgeons with lots of specimens sent for pathologic evaluation. Onco-pathology diagnostic center is the only pathologic diagnostic center providing immunohistochemical testing and the largest pathologic diagnostic center in the country with large number of pathologic specimens including breast tissue coming from the different corners of the country. Addis Hiwot General Hospital is among one of the private hospitals in the capital providing general and subspecialty surgical services and has its own dedicated pathology service which include histopathologic evaluation

4.9 Data Collection tools

Using data extraction checklist prepared by modification of different literature, which contains demographic data, histopathologic reports such as tumor size, histologic breast carcinoma subtype, Nottingham grade, ER, PR, HER-2 and Ki-67 index. The important data was collected from electronic record of Addis Hiwot General Hospital, Onco-pathology Diagnostic Center and chart review from SPHMMC medical record room.

4.10. Variables

4.10.1 Dependent variables (Outcome variable)

Intrinsic subtype of breast cancer

4.10.2 Independent variables

Age

Histo-pathologic findings of breast carcinoma. (Tumor histotype, grade, tumor size, stage, lymph node status, lymphovascular invasion, perineural invasion)

4.11 Quality measurement

To keep the reliability of the data, the principal investigator pretested research tool on the 10% of sample and training was given for data collector. During data collection completeness of research tool and accuracy was supervised by principal investigators. Kobotool box software was used for data entry.

4.12 Data presentation

Graphs, tables and diagrams are used to present findings.

4.13 Data processing and analysis plan

After data collection, it was exported to and analysed using SPSS 27th version. Descriptive statistics like frequencies and cross tabulation was made for most selected variables. Multiple logistic regression analysis was used to assess the association between the dependent variable and each independent variable. Multi variant analysis will be done to assess the strength of association and control confounding effect.

4.14 Operational definition

Molecular/ intrinsic breast carcinoma subtype- defined by ER, PR, HER-2 and KI-67 index

ER positive: tumor is estrogen receptor-positive if **at least 1% of the** cells tested have estrogen

PR positive: tumor is progesterone receptor-positive if **at least 1% of the** cells tested have progesterone receptors

Hormone receptor-positive: If the cancer cell has one or both of the receptors above, the term hormone-receptive positive (also called **hormone-positive** or **HR+**) breast cancer may be used.

Hormone receptor-negative: If the cancer cell does not have the estrogen or the progesterone receptor, (also called **hormone-negative** or **HR-**).

HER-2 positive: +2/+3

HER-2 negative: 0/+1

High KI-index: >20%

4.15 Ethical considerations

Ethical clearance was obtained from the Institutional review board (IRB) of the college after approval of the proposal. Confidentiality was kept by avoiding recording of names and potential identifiers of clients on data collection format, client records were made available to the investigators and data collectors and returned to its place after completion of data entry.

4.16 Dissemination of results

The result of the study will be presented to the community of SPHMMC and will be disseminated to concerned stake holders in order to enhance appropriate intervention and guide future plan for appropriate management of breast cancer cases. Finally, after rigorous review it will be published in reputable medical journals.

5. Results

In this study most of the cases were from Oncopathology diagnostic center(78%) followed by SPHMMC which comprises 17%. Data from Addis Hiwot General Hospital accounts for only 4.9% of cases. CNB was the most common type of procedure (48%) followed by mastectomy which accounted for 40%. Excisional and incisional biopsy comprises 5.5% and 4.1% respectively. Type of procedure was not specified for 11 cases.

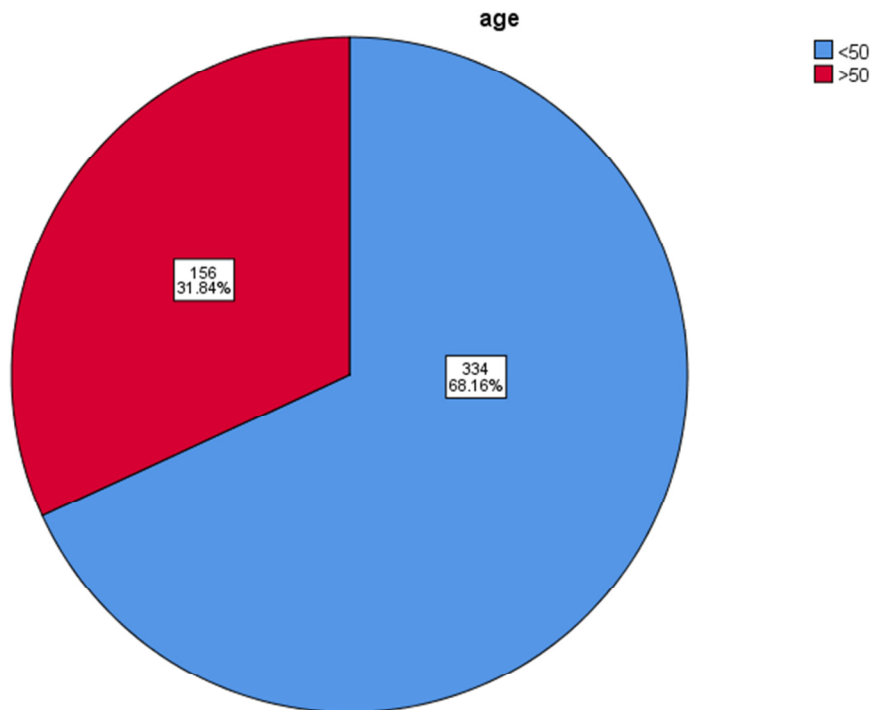


Figure 1: Age of breast cancer patients with histopathologic and breast cancer biomarker results in SPHMMC, Oncopathology diagnostic center and Addis Hiwot General Hospital from October 2023 to October 2024.

Majority (68.5) of the patients were below 50 years old. The average age of patients with breast cancer in this study was 45 years.

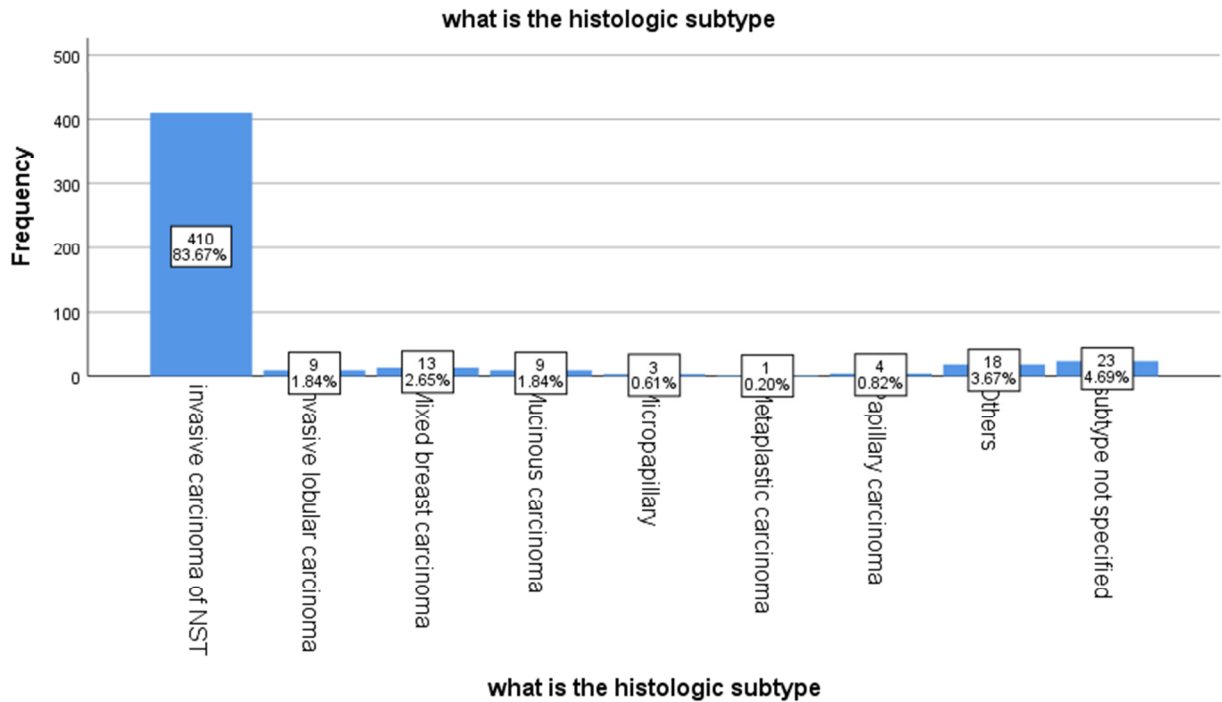


Figure 2: Histologic subtype of breast cancer cases with histopathologic and breast cancer biomarker results in SPHMMC, Oncopathology diagnostic center and Addis Hiwot General Hospital from October 2023 to October 2024.

Regarding the histologic sub type 410(83.6%) cases have invasive carcinoma of no special type followed by mixed carcinoma of invasive lobular and ductal carcinoma.

Table 1: Histopathologic variables of breast cancer cases with histopathologic and breast cancer biomarker results in SPHMMC, Oncopathology diagnostic center and Addis Hiwot General Hospital from October 2023 to October 2024.

Variables	Response	Frequency	Percentage
DCIS	Yes	121	24.7
	No	78	15.9
	Unknown	291	59.4
LCIS	No	110	22.4
	Unknown	380	77.6
Lymph node	Yes	135	27.6
	No	76	15.5
	Unknown	279	56.9
Lymph vascular invasion	Yes	174	35.5
	No	70	14.3
	Unknown	246	50.2
Perineural invasion	Yes	51	10.4
	No	119	24.3
	Unknwon	320	65.3
Pathologic stage	T1	50	10
	T2	114	23.1
	T3	44	8.9
	T4a	10	2.0
	T4b	10	2.0
	T4d	4	.8
	Unknown	258	52.2
Nottingham grade	Grade 1	27	5.5
	Grade 2	123	24.9
	Grade 3	46	9.3
	Uknown	293	59.3
Tumor size	Mean	4	

Out of 490 cases the presence or absence of DCIS is not documented in majority (60%) of the cases. DCIS was present in 25% and absent in 15% of the cases. Lymph node status was documented in 211 cases and 135 of them had Lymph node involvement. Two hundred forty-four cases had documented lympho-vascular space invasion status. Of these 174 had lympho-vascular space invasion. Perineural invasion status was known for 170 cases and only 51 cases had positive perineural invasion. Pathologic stage was not specified in 52% of the cases anthose cases with known .Fifty two percent of the cases had Regarding the Nottingham grading, most were having unknown status. Of the cases with known grading 123(25%), 46(9.3%), and 27(5.5%)had intermediate, high grade and low grade respectively.

Table 2: Biomarker status of breast cancer cases in SPHMMC, Oncopathology diagnostic center and Addis Hiwot General Hospital from October 2023 to October 2024.

Breast cancer biomarkers	Status	frequency	Percentage
Estrogen receptor	Positive	401	81.8
	Negative	86	17.6
	Unknwon	3	.6
Progesterone receptor status	Positive	354	72.2
	Negative	132	26.9
	Unknown	4	.8
HER-2	Positive	114	23.3
	Negative	346	70.6
	Unknown	30	6.1

Out of 487 cases with known ER status, 401(82.3%) had positive estrogen receptor while 86(17.7%) cases were negative for ER. Of 486 cases with known PR status, 354(72.8%) were PR positive while 27.2% were negative for PR. Among 460 cases with known HER-2 status 114(24.8%) express HER-2 and the rest 75.2% were negative

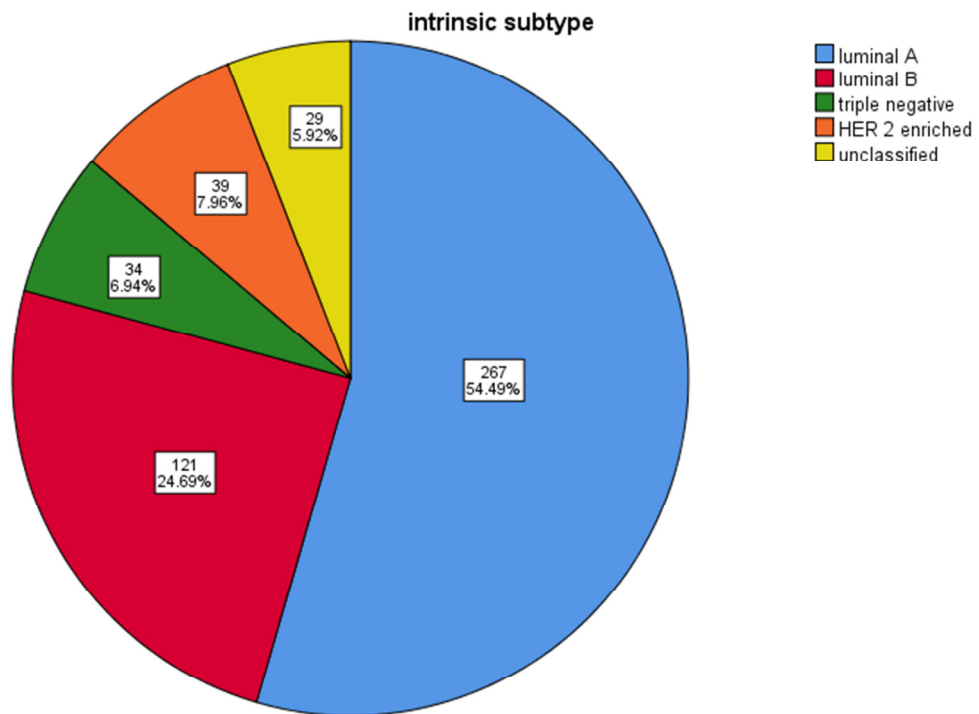


Figure 3: Intrinsic subtype of breast cancer cases in SPHMMC, Oncopathology diagnostic center and Addis Hiwot General Hospital from October 2023 to October 2024.

Four hundred sixtyone breast cancer cases were able to be classified based on biomarker status. Accordingly, the commonest intrinsic subtype was luminal A which accounted for 267(49%) cases followed by luminal B (25%). HER- 2 enriched breast carcinoma subtype was the 3rd common that accounted for 7.96%. Triple negative breast carcinoma was the least common subtype which accounted for 6.94%. Twenty-nine cases were not classified because of incomplete biomarker status. Ki 67 proliferation index was identified for only 65 cases and 59 of these were having KI 67 index greater than 20%.

Histopathologic and intrinsic subtype correlation

The Chi-square test was done to identify significant associations between patient age, tumor size, Nottingham grade, T stage, lymph node involvement, lymphovascular invasion and breast cancer intrinsic subtype on 148 cases that had complete histopathologic as well breast cancer biomarker tests. Age($p=0.018$), tumor size($p=0.007$) and Nottingham grade($p=0.00$) have shown significant association with the intrinsic subtype of breast carcinomas while carcinoma histologic subtype($p=0.704$), lymph node involvement ($p=0.984$) , lympho-vascular space invasion ($P=0.303$) and stage($p=0.102$) have not shown significant association with the intrinsic subtype of breast carcinoma.

Multinomial logistic regression was performed to determine how age, tumor size and Nottingham grade affects breast carcinoma intrinsic subtype. A total of 148 cases were used in the analysis.

Table 3: Multinomial logistic regression of histopathologic parameters and intrinsic subtype of breast cancer cases at SPHMMC, Oncopathologic diagnostic center and Addis Hiwot General Hospital from October 2023 to october 2024.

intrinsic subtype	Variables	95%CI AOR			P=value
Luminal B	Age of the patient in years	0.99	.97	1.03	.972
	largest diameter of the tumor	1.050	.875	1.261	.598
	[Is there Lymph node involvement=Yes]	2.6	.62	10.5	.19
	[Is there lympho-vascular space invasion=Yes]	.547	.119	2.519	.438

	[What is the Nottingham grade of the tumor=1]	.266	.059	1.197	0.044
	[What is the Nottingham grade of the tumor=2]	.364	.125	1.059	0.064
Triple negative	Age of the patient in years	.928	.829	1.038	.000 .191
	largest diameter of the tumor	.735	.369	1.463	.381
	Nottingham grade=2	.529	.432	.031	5.916
HER 2 enriched	Age of the patient in years	.939	.884	.997	.038
	largest diameter of the tumor	1.241	1.013	1.520	.037
	[Is there Lymph node involvement=Yes]	4.132	.234	72.992	.333
	[Is there lympho-vascular space	.096	.005	1.768	.115
	[What is the Nottingham grade of the tumor=1]	.003	.017	.001	.245
	[What is the Nottingham grade of the tumor=2]	.000	.039	.007	.210

As compared with luminal A the chance of luminal B breast cancers to be well differentiated is lower by 73.4%. As age increases by 1 year HER-2 enriched breast cancer decreases by 6.1%. As the diameter of tumor increases HER-2 enriched breast cancer cases increase by 1.24 times

6. Discussion

The mean age at diagnosis of breast cancer in this study is found to be 45.9 years which is comparable with metanalysis done in sub-Saharan countries which is 47.5 yrs and studies done in Saudi Arabia (49 yrs) and Iraq (48 yrs) (18,21,28).

Invasive carcinoma of NST is the commonest histologic subtype in this study accounting for 410 (83.67%) cases among the total 490 cases which is consistent with result in Saudi-Arabian study where among 740 BC cases 629(85%) were ductal carcinoma (28).

Out of 487 cases with known ER status, 401(82.3%) had positive estrogen receptor while 86(17.7%) cases were negative for ER. Of 486 cases with known PR status, 354(72.8%) were PR positive while 27.2% were negative for PR. Among 460 cases with known HER-2 status 114(24.8%) express HER-2 and the rest 75.2% were negative. The result has similarity with a metanalysis done in five Sub-Saharan African countries where ER+ was the predominant BC receptor (55%) among which in pooled proportion Ethiopia had the highest ER positivity (62%) and the HER-2 expression rate was found to be 23 % in this metanalysis. A retrospective cohort study done in Saudi-Arabia also has consistent result with 70.8% ER+, 63.8% PR+ and 18.7% HER-2+ among 740 BC cases. Where as in a study done in Ethiopia between 2015 and 2021 from 4 different regions half of the specimens tested negative for ER/PR among 201 cases and research done in Kijabe, Kenya from 2001-2007 in 120 BC cases show low ER positivity (24%). This difference from our study could be because of smaller sample sizes used in these two studies(15,17,18,28).

Four hundred sixty-one breast cancer cases were able to be classified based on biomarker status. Accordingly, the commonest intrinsic subtype was luminal A which accounted for 267(57.9%) cases followed by luminal B (26.2%). HER 2 enriched breast carcinoma subtype was the 3rd common that accounted for 8.4%. Triple negative breast carcinoma was the least common subtype which accounted for 7.37%. Ki 67 proliferation index was identified for only 65 cases and 59 of these were having KI 67 index greater than 20%. The result has consistency with metanalysis and scoping review done in different regions of Africa and among 24 countries where the pooled analysis among northern African countries had the highest luminal carcinoma

while the western African countries had the highest triple negative breast carcinoma and Ethiopia has the lowest triple negative breast carcinoma as per our result which makes different from most of African studies. With the same fashion in a comparative study done in USA among African American, white American, west African and Eastern African/Ethiopian patients with breast cancer, the Ethiopians had lowest rate of TNBC with 15% like that of the white Americans with 15.5% which was partly explained by population migration pattern during slave trade and ancestral sharing between west Africans and African American and between Ethiopians with mid East and central and south African regions. The finding was similar to the study of 334 breast cancer patients from different hospitals in Addis Ababa, Ethiopia 104 samples (31.1%) were luminal A, 91 samples (27.2%) were luminal B, 62 samples (18.6%) were HER2-enriched and 77 samples (23.1%) were triple negative. Similarly, the result from Saudi-Arabia shows the same pattern where luminal A was the most prevalent subtype (n = 434, 58.5%). In 2010 a population-based cancer registry in USA luminal A was the commonest intrinsic subtype where it accounted for 36810 (72.7%) among 50571 registries. Whereas study done in Ethiopia among 163 cases show triple negative as the commonest (33.1%) followed by Luminal B (27.6%). A prospective study in Iraq among 251 female breast cancer patients most of them (48%) were triple negative (15,19,21,23,27–29).

In this study age($p=0.018$), tumor size($p=0.007$) and Nottingham grade($p=0.00$) have shown significant association with the intrinsic subtype of breast carcinomas. As compared with luminal A the chance of luminal B breast cancers to be well differentiated is lower by 73.4% (AOR=0.266: (95%CI 0.059-1.197; $P=0.044$)) which is similar to study done on 334 cases in Addis Ababa, Ethiopia. As age increases by 1 year HER-2 enriched breast cancer decreases by 6.1%(AOR=.939(95%CI:.884-0.997)). HER-2 positive breast cancers have larger tumor diameter compared to HER-2 negative tumors (AOR=1.241(95%CI: 1.013-1.520). this result has similarity to study done in 4 regions of Ethiopia between 2015-2021 GC and research in Saudi-Arabia(15,28,29).

7. Limitation

As most of the cases were core needle biopsy it has incomplete histopathologic report which results in challenge during data analysis and makes difficult for regression and correlation of variables.

The patient data is not well integrated between Electronic medical record and medical record charts in SPHMMC and some of the records lack histopathologic and IHC report

Incomplete histopathologic and IHC report from pathologists

8. Conclusion

The mean age at diagnosis of breast cancer was 45 years. The most common histologic subtype was invasive carcinoma of NST. Majority of cases were positive for ER (82.3%) and PR (72.8%) and 75.2% of cases were HER-2 negative. Luminal A accounts for 57.9% cases followed by luminal B (26.2%). Triple negative breast carcinoma was the least common subtype which accounted for 7.37%. Age, tumor size and Nottingham grade have shown significant association with the intrinsic subtype of breast carcinomas. HER-2 positive breast cancer tends to occur in younger age and to have larger tumor size.

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8. Annex

8.1 Assurance of Principal Investigator

I the undersigned agree to accept all responsibilities for the scientific and ethical conduct of the research project. I will provide timely progress report to my advisor and seek the necessary advice and approval from my primary advisors in the course of the research. I will communicate timely to my advisors all stakeholders involved in the study including any source of funding for this research.

Name of the student: _____

Signature: _____

Date: _____

Approval of the Advisors

Name: _____

Signature: _____

Date: _____

Name: _____

Signature: _____

Date: _____

